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BROMOBENZENE TO THE DOG ON THE
PARTITION OF URINARY SULFUR

By ABRAHAM WHITE

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIRE-
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THE METABOLISM OF SULFUR

XIX. THE DISTRIBUTION OF URINARY SULFUR IN THE DOG AFTER THE ORAL ADMINISTRATION OF MONOBROMOBENZENE AS INFLUENCED BY THE CHARACTER OF THE DIETARY PROTEIN AND BY THE FEEDING OF *l*-CYSTINE AND *dl*-METHIONINE

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The synthesis of a phenylmercapturic acid after the administration of the monohalogen substitution products of benzene has been studied more extensively in the dog than in other animals (1). The most conclusive proof of this synthesis is to be obtained by isolation of the acid from the urine, but unfortunately this procedure is far from quantitative and isolation experiments give little information as to the extent of the synthesis. Since the sulfur of the molecule of the mercapturic acids is in organic combination, an approach to the quantitative aspect of this problem is available in the study of the partition of the urinary sulfur after the administration of the halogen derivative of benzene. If no compounds containing sulfur in unoxidized form other than the phenylmercapturic acid derivatives are excreted, such a study should give information of value; if other organic sulfur compounds are formed and excreted in the urine also, the evidence afforded by the sulfur partition studies must be interpreted with caution. Hele in experiments with dogs (1) and one of us (L.) in experiments with rabbits (2) have recently utilized this method of study. Other recent observers (3-7) have preferred for the most part to rely on the isolation of the mercapturic acid in the study of the problem.

It has generally been assumed that the amount of cystine available in the organism influences the extent of the synthesis of the mercapturic acids, but there exists some difference of opinion as

to whether cystine of endogenous origin may be utilized for purposes of synthesis as effectively as the cystine of the diet (either cystine liberated from protein in the processes of digestion or cystine fed as such). Thomas and his coworkers (3, 4) have held that the processes of endogenous protein catabolism do not result in the liberation of cystine or cysteine readily available for the formation of mercapturic acid after the administration of the monohalogen derivatives of benzene, since they were unable to isolate mercapturic acid from the urine of dogs fed bromobenzene and fasted or fed a protein-free diet, an observation confirmed by Muldoon, Shiple, and Sherwin (5). Abderhalden and Wertheimer (6) have criticized these experiments of Thomas because of the large amounts of bromobenzene fed to dogs weakened by prolonged fasting, and have obtained with shorter periods of fasting and smaller amounts of the halogen derivatives of benzene evidence of synthesis in fasted dogs, in fasted phlorhizinized dogs, and in dogs on a protein-free diet. They believe that the cystine requirements for reactions more essential than the detoxication of bromobenzene limit the extent of synthesis. The formation of phenylmercapturic acids in fasting rabbits has been demonstrated by Nishimura (7).

In most of the studies reported, the nature of the protein of the diet has had relatively little consideration. It appeared probable to us that more convincing evidence of the rôle of protein and of cystine in the synthesis of the mercapturic acids should be obtainable if the basal diet were adequate but of low protein content, particularly if the protein were so chosen that cystine was the limiting factor of the diet. We have studied the changes in the partition of urinary sulfur after the feeding of moderate amounts of bromobenzene to the dog in experiments in which casein and lactalbumin, as representative proteins of low and high content of cystine, supplied the protein element. We have also made similar studies in dogs fed a diet in which the protein was furnished by dried split peas, a diet known to be deficient in its cystine content (8), and have supplemented this diet by the addition of cystine. In view of recent observations (9, 10), which had indicated that methionine was able to replace cystine in the promotion of growth of white rats fed a diet in which an inadequate supply of cystine was the limiting factor, we have investigated the influence of this

other sulfur-containing amino acid on the mercapturic acid synthesis in the dog.

EXPERIMENTAL

Female dogs, accustomed to the routine of metabolism experiments, were used in these studies. The animals were kept in the usual metabolism cages and catheterized at regular 24 hour intervals. Immediately after catheterization, the daily food ration was given. The appetite of one of the dogs was little affected by the bromobenzene administration and for the most part, this animal consumed the diet regularly within 30 minutes after the

TABLE I
*Composition of Basal Diets**

	Diet A	Diet B	Diet C
	gm.	gm.	gm.
Casein.....	31.5		
Lactalbumin.....		29.0	
Dried peas.....			65.0
Sucrose.....	29.0	29.0	25.0
Lard.....	14.0	14.0	
Butter.....	14.0	14.0	25.0
Salt mixture (Karr)†.....	2.0	2.0	2.0
Tricalcium phosphate.....	4.0	4.0	4.0

* The dog received 0.9 gm. of Yeast Vitamine-Harris Powder in a gelatin capsule (No. 00) daily.

† Hawk, P. B., and Bergeim, O., *Practical physiological chemistry*, Philadelphia, 10th edition, 695 (1931).

food was offered. The greater number of experiments were therefore conducted with this animal. On the few occasions when the dog exhibited a reluctance to eat, forced feeding was resorted to, a procedure easily and quantitatively carried out with this dog. All of the data presented in Tables II to V were obtained with this animal, a long haired mongrel bitch of about 9.5 kilos weight. Similarly planned experiments were carried out also with a second dog with results comparable to those presented.

The diets, recorded in Table I, supplied protein from three sources, commercial casein, a commercial purified lactalbumin, and split peas (green). Each of these materials was analyzed for

nitrogen and sulfur and each was examined also for the presence of inorganic sulfate sulfur. The protein of Diets A and B supplied 4.01 gm. of nitrogen, while the peas of Diet C furnished 2.64 gm. of nitrogen. The sulfur supplied by the protein foodstuffs of the three diets was 0.380, 0.424, and 0.140 gm. respectively. The commercial casein and the peas were found on analysis to contain inorganic sulfate sulfur in amounts which, when deducted from the total sulfur of the protein foodstuffs of Diets A and C, gave organic sulfur values of 0.274 and 0.092 gm. respectively for these diets. Cystine determinations on the peas by the Folin-Marenzi (11) method indicated 0.43 per cent of cystine. The daily ration of peas therefore supplied about 0.280 gm. of this amino acid. The calorific value of the diets was such that the dog received the equivalent of slightly over 50 calories per kilo of body weight.

In the preparation of Diet C, the peas as purchased were finely ground in a mill and sieved repeatedly to insure a uniform product. This powder was weighed out, mixed with 100 cc. of water, and heated on a steam bath for 30 minutes. The mixture was then cooled and the other ingredients of the diet were incorporated to give a uniform mixture. The yeast vitamin concentrate was fed separately from the rest of the diet in a small gelatin capsule (No. 00). The additional nitrogen and sulfur of the diet from the vitamin and capsule were 95 and 15 mg. respectively. Bromobenzene, cystine, glycine, and methionine were given likewise in gelatin capsules immediately after catheterization except in one series of experiments (Table III) in which it was desired to observe the effect of dividing the intake of cystine and administering it in smaller amounts at intervals.

The usual analytical methods were employed, the Kjeldahl-Gunning method for total nitrogen, the Folin colorimetric method for creatinine, and the gravimetric methods of Folin and of Benedict as modified by Denis for the partition of sulfur. Creatinine determinations were made as a check on the completeness of collection of the 24 hour specimens of urine; and since uniform values were obtained from day to day, the creatinine figures are not presented in the tables.

DISCUSSION

The data obtained in experiments in which casein and lactalbumin were fed as the protein components of the diet are presented

in Table II. Although casein does not differ significantly from many common proteins in its content of total sulfur, its cystine content is very low, 0.3 to 0.4 per cent (12). Lactalbumin, on the other hand, contains more cystine than any other of the common proteins except the keratins, values of from 2.60 (13) to 4.25 (14) per cent having been reported.

When casein was fed, the oral administration of moderate amounts of monobromobenzene resulted in a greater excretion of

TABLE II

Excretion of Sulfur and Nitrogen after Oral Administration of Monobromobenzene As Influenced by Variation in Cystine Content of Protein of Diet

The initial weight of the dog was 9.0 kilos; the final weight, 8.3 kilos. An interval of 11 days elapsed between the experiments recorded.

Experiment 1. Casein diet (Diet A)							Experiment 2. Lactalbumin diet (Diet B)					
Day	Total N	Total S	Total sulfate S	Inorganic sulfate S	Ethereal sulfate S	Organic S	Total N	Total S	Total sulfate S	Inorganic sulfate S	Ethereal sulfate S	Organic S
	gm.	mg.	mg.	mg.	mg.	mg.	gm.	mg.	mg.	mg.	mg.	mg.
1	3.44	214	171	159	12	43	3.04	253	213	197	16	40
2	3.18	201	171	169	2	30	2.83	240	200	194	6	40
3	3.14	212	179	173	6	33	2.99	258	212	199	13	46
4	3.53	285	174	117	57	111*	3.15	264	162	118	44	102*
5	4.20	305	176	118	58	129*	3.41	290	161	117	44	129*
6	4.24	267	189	110	79	78*	3.55	284	142	97	45	142*
7	3.82	226	161	144	17	65	3.55	302	170	127	43	132*
8	3.55	230	180	169	11	50	3.33	270	189	162	27	81
9	3.21	222	178	165	13	43	3.26	260	203	193	10	57

* The animal received daily 1 gm. of monobromobenzene orally.

ethereal sulfate sulfur, an excretion which increased progressively and reached its maximum value on the 3rd day of bromobenzene feeding. Associated with this increase in the urinary ethereal sulfates was a rise in the organic sulfur excretion, which fell sharply on the last day of the administration of the benzene derivative. Similar results were obtained in another experiment not detailed in the tables in which, on the days when bromobenzene was fed, the ethereal sulfate and organic sulfur fractions of the urine were 48, 49, and 64 and 102, 110, and 56 mg. respectively.

If the organic sulfur excretion is an accurate index of the extent of mercapturic acid synthesis, these experiments would indicate that on a low cystine diet, less cystine is available for synthesis after repeated feedings of bromobenzene and that less of the benzene derivative is excreted as the mercapturic acid and more in conjugation with sulfuric acid. In Experiment 1 (Table II) the average daily "extra" excretions for the 3 day bromobenzene period were 56 mg. of ethereal sulfate sulfur and 66 mg. of organic sulfur. When the protein element of the diet was supplied by lactalbumin (Experiment 2, Table II), a protein of greater cystine content, the excretion of ethereal sulfate sulfur was increased somewhat following the feeding of the bromobenzene, but no greater excretion was observed at the end of the bromobenzene period than at the beginning. The organic sulfur excretion rose to a higher level than when casein was fed and showed no tendency to decline with continued bromobenzene feeding as on the casein diet. Although the total extra sulfur excreted in response to the administration of bromobenzene was approximately the same as that observed on the casein diet, the distribution was different, more extra sulfur appearing in the organic sulfur fraction (an average of 84 mg. daily as compared with 66 mg.) and less as ethereal sulfate sulfur (32 and 56 mg. on the lactalbumin and casein diets respectively).

The effect of the administration of bromobenzene on the elimination of nitrogen was different in the two experiments. When casein was the source of protein, the toxicity of the bromobenzene, as evidenced by the increased nitrogen elimination, was greater than when the lactalbumin diet was fed. We believe that this difference in the nitrogen excretion is related to the amount of cystine supplied by the diet and available for conjugation. When dietary cystine is not available in adequate amounts, the protein of the tissues is catabolized to furnish cystine for mercapturic acid synthesis and the level of nitrogen excretion is increased. Further evidence in support of this view was obtained in experiments in which the casein diet was supplemented by cystine. In these experiments the distribution of urinary sulfur resembled that observed in the lactalbumin experiment; *i.e.*, a greater increase in the organic sulfur fraction and no change in the ethereal sulfate sulfur after the 1st day of the administration of bromobenzene.

The increase in nitrogen elimination was less marked in these studies also. Since more striking evidence of this supplementary action of cystine was obtained in experiments in which the basal diet was lower in sulfur content (Diet C), these studies of the supplementary effect of cystine added to the casein diet are not reported in detail in this paper.

Since the experiments with casein and lactalbumin had indicated that the type of protein fed influenced the urinary sulfur distribution and presumably the mercapturic acid synthesis after the administration of bromobenzene, attempts were made to devise a basal diet even lower in its sulfur content than the casein diet and low in cystine. The low sulfur content of the proteins of the pea has long been known (15) and later studies have shown that the value of these proteins in nutrition is limited by the deficiency of cystine (8). It was considered of interest, therefore, to determine the effect of the ingestion of bromobenzene when the protein of the diet was furnished by dried green peas (Diet C), a diet which, in some experiments, was supplemented by the addition of cystine. The amount of cystine fed was usually the equivalent of 1.5 atoms of sulfur per molecule of bromobenzene (*e.g.*, Experiment 3, Table III) and in one experiment the equivalent of 2 atoms of sulfur (Experiment 4, Table III). In the earlier experiments, the cystine was fed in a single dose at the same time as the benzene derivative. It seemed probable, however, that the absorption of bromobenzene proceeded more slowly than that of the cystine. In later experiments, in order to insure a supply of cystine during the period of active metabolism of the bromobenzene, the cystine was fed in divided doses. The initial feeding occurred at the time of the administration of the bromobenzene and the remainder was fed at 3 hour intervals as indicated in Table III. The details of two such experiments are presented in Table III.

It will be observed that the urinary elimination of sulfur with Diet C (Table III) is very much less than in those experiments in which the dietary proteins were casein and lactalbumin (Table II), the total sulfur approximating 100 mg. on most of the normal control days. In contrast to the marked differences between the excretions of total sulfur and total sulfate sulfur on control days in Tables II and III, no variations of the organic sulfur, associated

with character of the protein component of the diet, are evident. This uniformity offers a striking illustration of the constancy of

TABLE III

Excretion of Sulfur and Nitrogen after Oral Administration of Monobromobenzene As Influenced by a Diet Low in Sulfur and Cystine Content (Diet C) with and without Added Cystine

The initial weight of the dog was 8.4 kilos; the final weight, 8.35 kilos. An interval of 25 days elapsed between the two experiments. During this interval, the animal was fed the same standard diet (Diet C) as during the experiments.

Day	Experiment 3						Experiment 4					
	Total N	Total S	Total sulfate S	Inorganic sulfate S	Ethereal sulfate S	Organic S	Total N	Total S	Total sulfate S	Inorganic sulfate S	Ethereal sulfate S	Organic S
	gm.	mg.	mg.	mg.	mg.	mg.	gm.	mg.	mg.	mg.	mg.	mg.
1	2.16	97	70	65	5	27	2.28	96	62	56	6	34
2	2.52	117	79	74	5	38	2.28	98	68	61	7	30
3	2.18	96	71	60	11	25	2.18	93	57	51	6	36
4	2.93	150	66	27	39	84*	2.95	153	76	29	47	77*
5	3.19	151	76	15	61	75*	3.01	133	45	6	39	88*
6	3.07	148	72	6	66	76*	3.13	142	60	2	58	82*
7	3.03	158	79	1	78	79*	3.37	162	70	0	70	92*
8	2.50	97	34	3	31	63	2.91	91	27	0	27	64
9	2.69	67	24	4	20	43	2.94	69	22	4	18	47
10	2.30	64	25	15	10	39	2.80	57	16	4	12	41
11	2.38	104	68	57	11	36	2.70	262	206	197	9	56†
12	2.30	226	126	80	46	100‡	2.34	376	250	208	42	126§
13	2.04	290	167	127	40	123‡	2.30	406	236	187	49	170§
14	2.16	315	178	119	59	137‡	2.62	431	245	187	58	186§
15	2.21	346	183	124	59	163‡	2.34	420	240	184	56	180§
16	2.41	185	100	75	25	86	2.25	144	67	47	20	77
17	2.35	100	62	53	9	38	2.25	114	64	51	13	50
18	2.38	100	66	56	10	34	2.28	103	63	53	10	40

* The animal received daily 1 gm. of monobromobenzene orally.

† The animal received 1.5 gm. of cystine orally, given in three doses at 3 hour intervals.

‡ The animal received daily 1 gm. of monobromobenzene and 1.15 gm. of cystine orally. The cystine was given in two doses at 3 hour intervals.

§ The animal received daily 1 gm. of monobromobenzene and 1.5 gm. of cystine orally. The cystine was given in three doses at 3 hour intervals.

the organic sulfur of the urine notwithstanding the variations of the sulfur and cystine contents of the diets, and emphasizes anew the endogenous character of this sulfur fraction.

The effects of the administration of bromobenzene on the sulfur elimination when Diet C (Table III) was fed were similar to, although more striking than, the casein experiment already discussed (Experiment 1, Table II). A marked rise in the excretions of both the ethereal sulfate and organic sulfur fractions of the urine occurred. These increased excretions were accompanied by a marked decrease in the inorganic sulfate sulfur fraction, a decrease which became so marked with the continued administration of bromobenzene as to result in the practical disappearance of this fraction of urinary sulfur from the urine. This effect was particularly noticeable in Experiment 4 (Table III), in which neither the total sulfur nor the inorganic sulfate sulfur of the urine had returned to their normal values on the 3rd day after the bromobenzene period. As in the casein experiments (Table II), the excretion of the ethereal sulfate sulfur increased progressively as the administration of bromobenzene was continued, while the organic sulfur showed little change after the 1st experimental day. For the 4 day experimental periods when bromobenzene was fed, the extra ethereal sulfate sulfur excretions were 208 and 178 mg. in Experiments 3 and 4 respectively, while the extra organic sulfur eliminations in the same periods were 174 and 199 mg. respectively. Accompanying these changes in the sulfur distribution, in each experiment a marked rise in the excretion of total nitrogen occurred. Although the excretions of total sulfur and inorganic sulfate sulfur were much depressed immediately after the administration of the bromobenzene was discontinued, the nitrogen excretions remained above the normal level for several days. As a result, the urinary N:S ratios were unusually high, *e.g.* 32, 42, and 49 on days 8 to 10 of Experiment 4, indicating a marked retention of sulfur without a corresponding retention of nitrogen.

When cystine supplemented Diet C in the periods of bromobenzene administration, the results were striking. Most notable was the absence of any increased urinary nitrogen despite the addition of about 134 mg. of cystine nitrogen to the diet. Thus in Experiment 4, on the 4th day of bromobenzene feeding (day 7), the nitrogen excretion was 3.37 gm. or about 1.0 gm. above the normal level. In the same experiment, when cystine was fed with the bromobenzene, the nitrogen excretion was 2.34 gm. on

the 4th day of the experimental period, or approximately normal. In a similar experiment not recorded in detail, the nitrogen eliminations of the 4th days of the bromobenzene experimental periods were 2.48 and 3.62 gm. with and without the addition of cystine to the diet. In still another experiment with a different and larger dog, the corresponding nitrogen excretions were 3.47 and 4.53 gm. respectively. In each of our experiments in which cystine has been added to supplement the low sulfur, low cystine protein of the peas, the increased nitrogen catabolism which invariably occurred after the administration of the bromobenzene alone has been absent. We believe that this effect on the level of nitrogen metabolism is convincing evidence of the specific rôle of cystine in the detoxication of bromobenzene.

Further evidence of this rôle of cystine in influencing the metabolism of bromobenzene may be obtained from a study of the partition of sulfur in the cystine-bromobenzene experimental periods. In Experiment 3, the extra ethereal sulfate and organic sulfur excretions were 168 and 383 mg. respectively for the 4 day experimental period (days 12 to 15). These figures may be contrasted with the corresponding values of 208 and 174 mg. of the period in which bromobenzene without cystine was fed (days 4 to 7). It will be noted that the elimination of extra ethereal sulfate sulfur was not increased by the addition of cystine; in fact, a slight decrease, possibly within the error of the experiment, appeared. The extra organic sulfur excretion was more than doubled when cystine supplemented the diet in the bromobenzene periods.

In Experiment 4 (Table III), cystine equivalent to 2 atoms of sulfur per molecule of bromobenzene was fed, a preliminary feeding of cystine was given prior to the administration of the bromobenzene in order to insure if possible an excess of cystine or its catabolites in the tissues, and the cystine was divided into three doses administered at 3 hour intervals. In this 4 day experimental period (days 12 to 15), the extra ethereal sulfate sulfur of the urine was not increased as compared with the period of bromobenzene feeding without cystine (169 and 178 mg. for the 4 day period with and without addition of cystine to the diet). The extra organic sulfur, 522 mg., was more than double that excreted in a similar period of the administration of bromobenzene alone 199 mg.

It was possible that some of this extra organic sulfur might have resulted from the administration of the cystine and have had no relation to the metabolism or detoxication of the bromobenzene. However, in control experiments with this same dog, in which cystine in amounts comparable to those of the preceding experiments was added to the basal diet (Diet C) in the absence of bromobenzene, we have never obtained an excretion of more than 60 mg. of extra organic sulfur in a 4 day period. Moreover, on those days in which cystine was fed in Experiments 3 and 4 and in other similar experiments, tests for cystine by the delicate cyanide-nitroprusside reaction in the urines have never indicated the presence of more than traces of the amino acid. While we regret the lack of an entirely satisfactory quantitative method for the determination of cystine in these urines, we consider it highly improbable that the high values for the extra organic sulfur in the cystine-bromobenzene periods are due to the presence of any significant amounts of cystine or the normal products of its catabolism.

In view of the interest in the recently discovered sulfur-containing amino acid of the protein molecule, methionine, and its possible importance as a supplement to or a substitute for cystine in nutrition (9, 10), we have studied the effects of the addition of *dl*-methionine (synthetic) to the basal pea diet (Diet C) on the urinary sulfur distribution when bromobenzene was fed. We have been unable to carry out as extensive studies as we have desired, since our supply of methionine was limited. We present, however, in Table IV the results of a series of such experiments with one of our animals. Since our knowledge of the fate of methionine in the dog was limited (15), methionine was fed without bromobenzene in a preliminary period (days 5 to 7). There occurred a marked depression of the elimination of nitrogen similar to that previously observed by one of us (16) when cystine was added to a diet low in its content of sulfur and of cystine. This low elimination of nitrogen continued on the 1st day after the feeding of methionine was discontinued. The excretion of organic sulfur increased somewhat and the values for this sulfur fraction remained above the normal for several days. Inasmuch as a more detailed study of the intermediary metabolism of methionine is in progress in this laboratory, we shall reserve further discussion of the possible significance of these findings until a later date.

When methionine (1.5 atoms of sulfur for each molecule of bromobenzene) was added to the basal diet in the periods in which

TABLE IV

Excretion of Sulfur and Nitrogen after Oral Administration of Monobromobenzene As Influenced by a Diet Low in Sulfur and Cystine Content (Diet C) with and without Addition of Glycine and of dl-Methionine to Diet (Experiment 5)

The initial weight of the dog was 11 kilos; the final weight, 10.7 kilos.

Day	Total N	Total S	Total sulfate S	Inorganic sulfate S	Ethe-real sulfate S	Or-ganic S	Day	Total N	Total S	Total sulfate S	Inorganic sulfate S	Ethe-real sulfate S	Or-ganic S
	gm.	mg.	mg.	mg.	mg.	mg.		gm.	mg.	mg.	mg.	mg.	mg.
1	2.24	113	77	63	14	36	21	2.23	157	110	99	11	47
2	2.28	108	72	57	15	36	22	2.16	151	110	100	10	41
3	2.28	106	79	68	11	27	23	2.63	208	124	90	34	84*
4	2.27	104	78	67	11	26	24	2.84	197	108	73	35	89*
5	2.09	192	146	134	12	46†	25	3.10	214	112	54	58	102*
6	1.81	271	225	218	7	46†	26	3.06	201	125	32	93	76*
7	1.86	297	236	230	6	61†	27	2.76	161	84	43	41	77
8	1.69	193	131	124	7	62	28	2.76	184	128	89	39	56
9	2.05	154	100	88	12	54	29	2.83	179	123	95	28	56
10	2.17	127	90	81	9	37	30	2.66	169	122	79	23	47
11	2.02	115	83	74	9	32	31	2.51	151	102	79	23	49
12	2.07	120	83	80	3	37	32	2.30	221	125	84	41	96‡
13	2.02	113	76	72	4	37	33	1.81	284	154	104	50	130‡
14	1.83	215	118	83	35	97‡	34	1.63	293	154	101	53	139‡
15	1.62	279	157	121	36	122‡	35	1.67	311	168	110	58	143‡
16	1.46	268	145	106	39	123‡	36	1.74	187	106	81	25	81
17	1.76	304	162	117	45	142‡	37	2.29	154	101	85	16	53
18	1.66	190	115	85	30	75	38	2.39	149	102	93	9	47
19	2.18	146	90	71	19	56	39	2.31	149	104	90	14	45
20	2.25	150	102	91	11	48							

* The animal received daily 0.72 gm. of glycine and 1 gm. of monobromobenzene orally.

† The animal received daily 1.4 gm. of *dl*-methionine orally.

‡ The animal received daily 1.4 gm. of *dl*-methionine and 1 gm. of monobromobenzene orally.

bromobenzene was fed (days 14 to 17 and days 32 to 35), the results resembled those of the experiments¹ in which cystine supplemented

¹ Further evidence of the similarity in the action of cystine and methionine in the detoxication of bromobenzene is to be found in the symptoms

the basal diet during bromobenzene administration (Table III). There was a marked depression of nitrogen excretion, the elimination of ethereal sulfate sulfur increased little after the initial rise of the 1st day of bromobenzene feeding, and there was an increased excretion of organic sulfur which reached its maximum value on the last day of the feeding period. The effects of methionine feeding were so remarkable that the question arose as to whether the changes observed were specific for the sulfur-containing amino acids or whether they might be due to the influence of any amino acid regardless of its sulfur content. As a further control, we administered glycine with the bromobenzene in amounts comparable to those of the sulfur-containing amino acids (days 23 to 26, Table IV). When glycine was fed, the sulfur and nitrogen excretions were quite comparable to those in the experiments in which bromobenzene was fed without the addition of an amino acid (Table III). The nitrogen excretion was increased; the ethereal sulfate sulfur increased progressively, reaching its maximum value on the 4th day of the bromobenzene period, while the rise in organic sulfur was less marked and the excretion declined sharply on the last day of the period. The nitrogen excretion during the initial control period of 4 days was 9.08 gm., a level which was maintained approximately throughout subsequent control periods. In the two periods in which methionine and bromobenzene were fed together, the nitrogen excretions during 4 days were 6.67 and 7.41 gm., respectively, while in the period in which bromobenzene and glycine were fed, the nitrogen elimination was 11.63 gm. These differences in nitrogen are striking and indicate that methionine administered with bromobenzene is as effective in preventing an increased level of nitrogen catabolism as is cystine.

The interpretation of the changes in organic sulfur is not easy. There occurred an unquestionably greater increase in the organic sulfur fraction of the urine when methionine was fed with bromobenzene than when glycine and bromobenzene were fed. The feeding of methionine alone resulted in some increase in the organic sul-

exhibited after the feeding of the bromobenzene. When the basal diet was fed alone or supplemented by glycine, the animal was depressed for several hours after the administration of the bromobenzene; when methionine or cystine was added to the basal diet, the toxic effect of the bromobenzene was clearly less marked.

fur of the urine. We do not believe, however, that the higher values for organic sulfur in the methionine-bromobenzene periods as compared with the similar values in the glycine-bromobenzene period can be explained entirely as a result of the ingestion of the methionine. The increases in extra organic sulfur are similar to those observed in the cystine experiments of Table III and other like experiments, although probably not so marked.

As stated previously, the changes in the organic sulfur fraction of the urine after the administration of monohalogen derivatives of benzene have been considered as quantitative evidence of the extent of synthesis of the phenylmercapturic acids. While we believe that such an interpretation of the data is probably justifiable, we hesitate to apply it to our own results and to conclude that the ingestion of methionine leads to a more extensive synthesis of a mercapturic acid or to the synthesis of some similar substance in which the methionine molecule or a product of its metabolism is concerned. Such a conclusion can be justified only when satisfactory methods for the quantitative determination of the mercapturic acids are available for the analysis of the urines after the feeding of benzene derivatives or when a product derived from the conjugation of methionine, or a derivative of this amino acid, with monobromobenzene shall have been isolated. For the present, it is sufficient to point out the similarity in the changes in the sulfur metabolism when either cystine or methionine is administered with bromobenzene and to suggest that methionine may function as does cystine in the detoxication of the monohalogen derivatives of benzene. It is possible also that cystine and methionine may have some common product of intermediary metabolism which is essential for the normal function of the organism and that when methionine is supplied by the diet, the cystine present is thereby made available for the detoxication of the benzene derivatives.

Certain observations made in connection with these experiments are of interest in relation to the problem of the origin and significance of the increased urinary ethereal sulfates after bromobenzene feeding. It has been pointed out that the level of ethereal sulfate excretion after the administration of bromobenzene appeared to be related to the amount of cystine available in the organism for the detoxication of the monohalogen benzene deriva-

tive. Whenever bromobenzene was given to the animal maintained on a dietary régime low in cystine, the excretion of ethereal sulfates increased progressively with the repeated daily doses of the toxic compound. In contrast to these results, when adequate cystine was supplied, either as the free amino acid or in the form of protein (lactalbumin), the ethereal sulfate excretion tended to rise to a level above the normal excretion but remained approximately at that level during the days of the feeding of monobromobenzene.

In view of these results, it appeared probable to us that whenever adequate amounts of cystine were available, the animal organism detoxified monobromobenzene by conjugation with the amino acid and excreted it as the bromophenylmercapturic acid. If a cystine deficiency existed, the animal was forced to resort to some other means of removing the toxic bromobenzene. A portion of the compound might be excreted as such and a portion might be either partially or completely oxidized. The first step likely to occur in the oxidation of bromobenzene would result in the formation of *p*-bromophenol. That the latter reaction may take place in the animal organism is not at all improbable. Under conditions of stress, drastic oxidations may occur in the animal organism, as is exemplified in the experiments of Jaffé (17), who was able to isolate small amounts of muconic acid from the urine of dogs which had been given benzene. This evidence for the cleavage of the benzene ring *in vivo* would support a hypothesis which assumes the much milder reaction; that is, the introduction of hydroxyl groups into the benzene nucleus. Conditions which favor the formation of these phenolic compounds should, therefore, augment the urinary excretion of ethereal sulfates. It is significant that throughout these experiments progressively increasing excretions of ethereal sulfates were noted when bromobenzene was administered to the animal maintained on a low cystine diet. An oxidation of bromobenzene to *p*-bromophenol, occurring when a cystine deficiency prevents detoxication of the halogen benzene derivative by the formation of a mercapturic acid, would increase the excretion of ethereal sulfates.

Further experimental evidence is presented in Table V, in which are given the details of an experiment in which *p*-bromophenol was fed with and without the addition of sodium sulfate to Diet C.

The administration of *p*-bromophenol resulted in an increased excretion of sulfur as ethereal sulfates and a marked decrease in the inorganic sulfate fraction, a decrease so marked as to result

TABLE V

Excretion of Sulfur and Nitrogen after Oral Administration of p-Bromophenol As Influenced by a Diet Low in Sulfur and Cystine Content (Diet C) with and without Feeding of Sodium Sulfate (Experiment 6)

The initial weight of the dog was 10.7 kilos; the final weight, 10.0 kilos.

Day	Total N	Total S	Total sulfate S	Inorganic sulfate S	Ethereal sulfate S	Organic S	Day	Total N	Total S	Total sulfate S	Inorganic sulfate S	Ethereal sulfate S	Organic S
	gm.	mg.	mg.	mg.	mg.	mg.		gm.	mg.	mg.	mg.	mg.	mg.
1	2.57	107	69	61	8	38	17	2.46	337	310	217	93	27*
2	2.54	109	78	71	7	31	18	2.35	378	350	249	101	28*
3	2.35	106	75	66	9	31	19	2.56	381	354	215	139	27†
4	2.53	105	77	71	6	28	20	2.45	365	338	192	146	27†
5	2.67	142	110	8	102	32‡	21	2.34	384	357	222	135	27†
6	2.70	133	105	0	105	28‡	22	2.10	97	70	61	9	27
7	2.72	134	105	0	105	29‡	23	2.11	94	67	63	4	27
8	2.67	139	110	0	110	29‡	24	2.04	98	71	63	8	27
9	2.51	48	21	0	21	27	25	2.09	98	72	63	9	26
10	2.53	46	22	11	11	24	26	2.45	140	117	6	111	23§
11	2.34	113	87	81	6	26	27	2.32	137	115	0	115	22§
12	2.35	109	76	68	8	33	28	2.32	138	110	0	110	28§
13	2.25	112	81	73	8	31	29	2.51	110	86	0	86	24§
14	2.31	289	261	258	3	28	30	1.93	37	14	2	12	23
15	2.40	296	267	259	8	29	31	2.04	49	24	14	10	25
16	2.46	380	352	254	98	28*	32	1.96	115	90	80	10	25
							33	2.04	99	74	64	10	25

* The animal received daily 1.22 gm. of sodium sulfate and 1.1 gm. of *p*-bromophenol orally.

† The animal received daily 1.22 gm. of sodium sulfate and 1.65 gm. of *p*-bromophenol orally.

‡ The animal received daily 1.1 gm. of *p*-bromophenol orally.

§ The animal received daily 1.65 gm. of *p*-bromophenol orally.

|| On days 14 and 15 the animal received 0.81 and 1.22 gm. respectively of sodium sulfate orally.

in the complete disappearance of this fraction of urinary sulfur. Further suggestive evidence that ethereal sulfates were being formed at the expense of inorganic sulfate sulfur during the period of the administration of the *p*-bromophenol was obtained

in experiments in which sodium sulfate was fed with the phenol. During those experimental days (days 19 to 22), there was a slightly increased excretion of ethereal sulfates as compared with the excretion when bromophenol was fed alone with the basal diet (days 26 to 29). The administration of the *p*-bromophenol did not lead to any alteration in the organic sulfur of the urine. Hence the suggestion that oxidation to the phenol is a preliminary step in the synthesis of *p*-bromophenylmercapturic acid after the administration of bromobenzene appears improbable. Further studies of this problem are in progress.

SUMMARY

1. The distribution of sulfur in the urine after the oral administration of monobromobenzene has been studied in dogs maintained on diets, the protein elements of which varied in their content of sulfur and of cystine.

2. Greater increases in the organic sulfur fraction of the urine after bromobenzene feeding were observed when the protein of the diet was furnished by lactalbumin, a protein rich in cystine, than when the protein element was supplied by casein or by peas, both low in cystine content. On these low cystine basal diets, the extra ethereal sulfate sulfur excretion after bromobenzene was greater than when a basal diet of higher cystine content (lactalbumin) was fed.

3. The addition of either *l*-cystine or *dl*-methionine to the low-cystine basal diet (peas) in the bromobenzene feeding periods prevented the increased nitrogen excretion in the urine which occurred after the administration of bromobenzene when the basal diet was fed without supplement. The sulfur distribution resembled that found in the experiments in which lactalbumin was the source of the dietary protein.

4. The effects of cystine and methionine on the nitrogen elimination and on the distribution of the urinary sulfur following bromobenzene feeding appeared to be specific since similar results were not obtained when glycine was fed with bromobenzene.

5. The relation of these changes in sulfur metabolism to the problem of the synthesis of the phenylmercapturic acids is discussed.

6. No increase in the organic sulfur of the urine was observed

to result from the feeding of *p*-bromophenol under conditions of a low cystine basal diet (peas). This is believed to support the theory of Coombs and Hele (18), who have suggested that two paths of catabolism of bromobenzene are open, the one the oxidation, conjugation, and elimination as ethereal sulfates, the other the synthesis of mercapturic acid. It is considered probable that if abundant cystine is available, the major portion of the bromobenzene is conjugated with the amino acid and excreted as the mercapturic acid, while, if the supply of cystine is limited, oxidation, conjugation, and elimination of the bromobenzene as ethereal sulfates is the predominant means of detoxifying the benzene derivative.

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